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Biomarkers of progestin therapy resistance and endometrial hyperplasia progression

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Abstract

Objective—To identify biomarkers associated with progestin therapy resistance and persistence/progression of endometrial hyperplasia.

Study Design—We performed a nested case-control study among women with complex (n=73) and atypical (n=41) hyperplasia treated with oral progestin, followed 2–6 months for persistence/progression. We evaluated index endometrial protein expression for progesterone receptors A (PRA) and B (PRB), PTEN, Pax-2 and Bcl-2. Odds ratios and 95% confidence intervals were estimated.

Results—Among women with atypical hyperplasia, high PRB expression was associated with 90% decreased risk of persistence/progression (95% CI: 0.01–0.8). High expression of PRA and PRB suggested decreased risk of persistence/progression (OR=0.1, 95% CI: 0.02–1.0). These findings were not observed among women with complex hyperplasia. No associations were found with PTEN, Pax-2, and Bcl-2 protein expression.

Conclusions—PRB expression shows promise as a biomarker of progestin response. Further research is warranted to understand how PRB expression may guide treatment decisions.

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Keywords

Biological Markers; Endometrial Hyperplasia; Immunohistochemistry; Progesterone Receptors; Progestin

Introduction

Endometrial carcinoma is the most common reproductive tract cancer in the United States.¹ Women with endometrial hyperplasia (EH), an abnormal proliferation of the endometrial glands,^{2–4} are at increased risk of developing carcinoma. EH is commonly managed with progestin therapy with surveillance or hysterectomy,^{5–7} depending on the severity of the histopathologic diagnosis and the perceived risk of progression to carcinoma. The World Health Organization (WHO) classifies EH severity as simple or complex, with or without cytological atypia, based on architectural and cytologic features.^{2, 8} The likelihood of progression to carcinoma ranges by EH severity from: simple hyperplasia without atypia (SH) - often spontaneously regresses;^{2, 3} to complex hyperplasia without atypia (CH) - rarely progresses to endometrial cancer;^{2, 3} to atypical hyperplasia (AH) - has the greatest risk of concomitant carcinoma⁹ and/or subsequent carcinoma.^{2–4, 10} Therefore, hysterectomy is generally recommended for women diagnosed with AH not desiring future fertility.^{5–7}

In this treatment paradigm, the medical and surgical treatment risks, costs, and quality of life issues differ substantially between a diagnosis of CH and AH. This is concerning given the low diagnostic reproducibility of the WHO classification scheme,^{11–15} particularly disagreement on key histologic features that distinguish AH from CH.¹⁵ Furthermore, new findings suggest that the majority of women with AH respond to progestin.^{10, 16} In our prior report, 26.9% of women with AH treated with 2–6 months of progestin had persistence/progression of endometrial hyperplasia as compared to 66.7% of those not treated (relative risk (RR) 0.39, 95% confidence interval (CI) 0.21–0.70).¹⁶ We found the long term risk of endometrial carcinoma among women with AH who used progestin was 20.5 per 1,000 woman-years as compared to 101.4 per 1,000 woman-years among non-users.¹⁰ Given the suboptimal reproducibility of WHO diagnostic criteria and the potential benefits of progestin therapy among women diagnosed with AH, there is a need for biomarkers that could predict progestin therapy resistance and EH persistence/progression, providing valuable information to guide treatment decisions.

Various immunohistochemical (IHC) biomarkers have been investigated for association with EH and/or endometrial carcinoma, in an attempt to distinguish between normal endometrium and endometrial neoplasm (pre-invasive hyperplasia and invasive carcinoma).¹⁷ Relatively few longitudinal studies have explored IHC biomarker expression and EH persistence/progression with progestin therapy.^{18–22} The goal of most of these studies was to evaluate the change in IHC biomarkers pre- and post-progestin therapy, with only one study seeking to identify baseline IHC biomarkers predictive of progestin therapy response in terms of EH progression.¹⁸

The purpose of this study was to identify IHC biomarkers associated with progestin therapy resistance, defined as EH persistence/progression among women with CH or AH treated with oral progestin. We chose to investigate biomarkers of protein expression for which there is some evidence of: 1) early involvement in endometrial neoplasia; 2) a proposed steroid hormone receptor expression relationship; and 3) proven endometrial immunohistochemical methodologies. Specifically, we assessed expression of progesterone

receptor isoforms A (PRA) and B (PRB), the tumor suppressor protein PTEN, the oncogenic protein Pax-2, and the anti-apoptotic protein Bcl-2.

Materials and Methods

Study Population and Design

We performed a nested case-control study among women treated with progestin from a cohort with an index consensus diagnosis of CH or AH using the WHO classification scheme^{8, 23} and followed 2 to 6 months for persistence/progression of EH. Detailed Endometrial Hyperplasia Cohort study methods have been previously reported.^{10, 15, 16, 24, 25} Briefly, the study was conducted among female enrollees at Group Health (GH), an integrated health plan in Washington State. Women over age 18 years diagnosed with CH and AH between January 1, 1985 and April 1, 2005 were identified and were eligible if they had at least one additional endometrial pathology specimen (biopsy or hysterectomy) taken 8 weeks to 6 months after the index diagnosis with baseline tissue blocks available. This time interval corresponds with common clinical practice of progestin treatment for 2 to 6 months with pathology re-assessment at treatment completion.^{5, 16, 26} Women were excluded if they had a hysterectomy, were diagnosed with endometrial cancer, or disenrolled from Group Health within 8 weeks of the index diagnosis. Histology slides were reviewed blindly, independently, and in random order by two University of Washington (UW) pathologists (R.L.G., K.H.A.) with a rigorous adjudication process.¹⁵ Pathology data were linked with automated enrollment, pharmacy, inpatient and outpatient clinical databases.

For this study, 217 women with CH (N=138) and AH (N=79), confirmed by consensus review, were initially considered for inclusion, pending data on progestin treatment. We received approval from the Group Health and Fred Hutchinson Cancer Research Center Institutional Review Boards prior to the conduct of the study.

Progestin treatment

Automated pharmacy data was obtained on all dispensings of any progestin from one week before the index biopsy up to 6 months after the index biopsy, but prior to follow-up biopsy. Women were only included if more than 14 days of progestin was dispensed. The pharmacy database provided progestin type, dose, and duration dispensed.

Outcome ascertainment

The outcome was dichotomized into persistence/progression and regression of CH or AH by comparing the diagnosis at follow-up with the index diagnosis. Cases were women whose CH or AH persisted/progressed, and controls were women whose CH or AH regressed. For example, among women with an index diagnosis of CH, follow-up diagnoses of CH, AH, or carcinoma were classified as CH persistence/progression and diagnoses of no hyperplasia or SH were classified as CH regression. We selected this outcome as failure to regress after 3 to 6 months of progestin therapy is commonly an indication for hysterectomy.^{5, 16, 26}

Exposure ascertainment, IHC methodologies

Exposures of interest were expression of PRA, PRB, PTEN, Pax-2, and Bcl-2 proteins as measured by IHC. IHC was conducted by the UW Immunohistochemistry Research Laboratory. IHC stains for each biomarker were performed on unstained slides cut from formalin fixed, paraffin embedded tissue blocks. All tissues were deparaffinized followed by blockade of endogenous peroxidases and antigen retrieval using Antigen Unmasking Solution (Vector; USA). Pretreatment, antibody selection, dilution, incubation time, and positive control varied for each protein (Table 1). The slides were counterstained in

hematoxylin. Negative controls for each run were conducted using normal mouse or rabbit serum instead of the primary antibody on tissue.

IHC was run in a single batch for all samples and the antibody staining was scored without knowledge of the index or follow-up diagnoses. Nuclear antigens PRA and PRB were scored based on degree of nuclear staining where as cytoplasmic antigen Bcl-2 was scored based on degree of cytoplasmic staining. PTEN is present in both cytoplasm and nuclei and both cytoplasmic and nuclear staining were considered in scoring. One of two study pathologists scored the percentage of cells with positive antibody staining, or for PTEN the loss of antibody staining, in the lesional tissue as 0%, 1–25%, 26–50%, 51–75%, or 76–100%.

We defined high expression of PRA or PRB and no loss of Pax-2 and Bcl-2 biomarker expression as >75% of cells staining positive. We defined low expression of PRA or PRB and loss of Pax-2 and Bcl-2 expression as <75% of cells staining positive. For PTEN, we defined no loss of expression as <25% loss of staining and loss of expression as >25% loss of staining. Cut points that were conservative with regard to high or no loss of expression were chosen to minimize misclassification of biomarker expression and were informed by review of the distribution of expression.²⁵

Covariate ascertainment

Age, race, parity, height, weight, personal history of breast or colon cancer, diabetes, and smoking status at index diagnosis, as well as oral contraceptive and postmenopausal hormonal therapy use in the 6 months prior to index diagnosis, were abstracted from the GH medical record.¹⁶

Statistical Analyses

To evaluate the relationship between individual biomarkers at index and the risk of EH persistence/progression at follow-up, we estimated the odds ratio (OR) as the measure of association and exact 95% CI using the `epitab` command in Stata 11.0 (STATA Corporation, College Station, Texas) and conducted Fisher's exact test with $\alpha=0.05$ significance level. Each IHC biomarker was analyzed as a dichotomous variable, high or low expression (PRA and PRB) and loss or no loss of expression (PTEN, Pax-2, Bcl-2). Analyses were performed separately among women with CH and AH and the role of age (<50 years, ≥ 50 years) and body mass index (BMI) (<30 kg/m², ≥ 30 kg/m²) as confounding factors in the association were evaluated. To assess if the association between individual biomarkers and progestin therapy resistance varies by duration of progestin therapy, we repeated the analyses among women with at least 8 weeks of progestin treatment.

Results

We excluded 77 of the 217 women with CH and AH considered for IHC analyses because: material was not available for IHC at index (n=54); histologic presentation of the IHC stained sample was different from the original diagnostic slide (n=4); or material was insufficient for accurate IHC scoring (n=19). We restricted our analyses to 114 women with CH (n=73) and AH (n=41).

Among women with an index diagnosis of CH, a greater proportion of cases than controls were younger (< 39 years), current smokers, and overweight (BMI ≥ 25 kg/m²). Similarly, among women with AH, cases tended to be younger (< 49 years), current smokers, and obese (BMI ≥ 30 kg/m²) compared to controls. Overall, cases were less likely than controls to be nulliparous. Among women with an index diagnosis of CH, the time interval between index and follow-up biopsies and weeks of progestin use, on average, were generally comparable

between cases and controls. While the time interval and weeks of progestin use were greater among controls compared to cases among women with an index diagnosis of AH, the percent time on progestin treatment, or the estimated proportion of weeks between index and follow-up diagnoses in which progestin was taken, was similar for both groups. The majority of women with an index diagnosis of CH (cases-61.9%, controls-75.0%, $P=0.271$) and AH (cases-75.0%, controls 89.7%, $P=0.334$) were treated with progestin for at least 8 weeks between index and follow-up biopsies. The type and dose of progestin treatment were similar between cases and controls with the exception that, among women with an index diagnosis of AH, controls were less likely than cases to receive low dose progestin treatment, defined as medroxyprogesterone acetate (MPA) <10mg/day or norethindrone acetate <1 mg/day (Table 2).

CH persisted/progressed at follow-up in 21 of the 73 women with an index diagnosis of CH treated with progestin: 2-adenocarcinoma, 8-AH, and 11-CH. Among the 41 women with an index diagnosis of AH treated with progestin, AH persisted/progressed in 12 women: 5-adenocarcinoma and 7-AH.

Among women with CH at index, no associations were found between PRA, PRB, PTEN, Bcl-2, and Pax-2 protein expression and persistence/progression. Among women with AH at index, high expression of PRB was associated with a 90% decreased risk of persistence/progression (95% CI: 0.01–0.8). Additionally, there was a suggestion of a decreased risk of persistence/progression with high expression of PRA and PRB compared to low expression of both receptors (OR 0.1, 95% CI: 0.02–1.0). No associations were found between PTEN, Pax-2, and Bcl-2 protein expression measured at index biopsy and AH persistence/progression (Table 3). When the analyses were repeated adjusting for age and BMI, the magnitude of the associations were similar. Additionally, when we repeated the analyses among women with at least eight weeks of progestin treatment, we observed the same pattern of associations as in the main analyses (data not shown).

Comment

To our knowledge, this is the first study to evaluate, separately by index diagnosis of CH or AH, the association between index biomarker expression and persistence/progression of EH among women treated with oral progestin (search: PubMed; terms: endom* hyperplasia, progest*, immunohistochemistry, adult, female; language: English; dates: June 1979 – January 2012). We found that among women with an index diagnosis of AH, high PRB expression, and possibly high expression of both PRA and PRB, were associated with a decreased risk of AH persistence/progression with progestin therapy.

The presence of progesterone receptor expression at baseline, leading to a decreased risk of EH persistence/progression following progestin therapy, makes clinical and biological sense. EH is stimulated by estrogen. Clinical research has demonstrated that unopposed estrogen therapy in postmenopausal women increases EH risk and that most revert with administration of progestin.²⁷ In our prior study, we found over a six month period that women treated with oral progestin had a 61% decreased risk of persistence/progression of AH as compared to women not treated (RR 0.39, 95% CI: 0.21–0.70).¹⁶ (The majority of women who took progestin in that study contributed to the analyses reported here). While the complex mechanism of progestin action at the molecular level is not known, progestin may potentiate EH regression by antagonizing estrogen-induced proliferation through decreased synthesis of estrogen receptors and/or promoting the enzymatic conversion of estradiol to estrone.^{28–30} Progestin may also act via estrogen-independent pathways.^{31, 32} These possible biological effects of progestin require the presence of progesterone receptors.

Thus, low progesterone receptor expression, particularly PRB, may identify a neoplastic subpopulation prone to progestin resistance.

Our finding is consistent with two studies of women with a baseline diagnosis of AH or endometrial endometrioid carcinoma treated with oral progestin. Utsunomiya et al³³ studied 16 women with endometrial endometrioid adenocarcinoma treated with MPA (600mg/day) for at least 6 months. Defining progestin responsiveness as regression to normal endometrium or hyperplasia without atypia, the investigators found that women who responded to MPA had greater expression of both PRA and PRB or an increased ratio of PRB/(PRB+PRA) at index.³³ An unpublished study conducted among women with AH or grade 1 endometrioid adenocarcinoma (G1EAC) who were treated with progestin for at least 8 weeks found that strong expression of PRB at baseline was associated with resolution of AH or G1EAC (definition of resolution not provided).³⁴

One additional study may have relevance to our findings among women with CH. Women with EH were treated for 3 months with oral MPA (6 women with CH, 8 with AH and 15 with SH) or the levonorgestrel intrauterine system IUS (21 with EH).²¹ Among those treated with MPA (not stratified by EH type), there was no difference in glandular or stromal expression of PRA or PRB at baseline, between women who regressed to normal proliferative or atrophic endometrium and women with persisting hyperplasia. Complete resolution of EH was noted in all women treated with the levonorgestrel IUS, regardless of PR status.²¹ Others have confirmed the association of PR and progestin therapy response with levonorgestrel IUS treatment of EH.^{18, 20} Akesson et al¹⁸ showed that among levonorgestrel IUS users, a greater proportion of the 29 women with CH and 5 women with AH who had PRA or PRB expression at baseline were more likely to resolve any form of EH, as compared to women without PRA or PRB expression; the median time interval for response was 9.9 months.

We did not find an association between baseline PTEN expression and EH persistence/progression. The *PTEN* tumor suppressor gene regulates epithelial growth by inhibiting cell proliferation and inducing apoptosis.³⁵ Progesterone has been demonstrated to increase PTEN protein levels by decreasing PTEN phosphorylation.³⁶ A small study of 31 women diagnosed with CAH or G1EAC treated with oral MPA, found that the presence of PTEN null glands at baseline may be suggestive of an increased risk of hysterectomy (performed if no change in diagnosis or carcinoma relapse after MPA treatment).³⁷ A second study, among women with predominantly CH treated with the levonorgestrel IUS, found no association of PTEN with EH progression.¹⁸ These inconsistent study findings may be due to small numbers, differences in subject selection, and/or determination of PTEN loss.

We also did not find an association between Pax-2 or Bcl-2 expression and progestin therapy resistance. While Pax-2 has not previously been investigated with regard to progestin therapy resistance, others have reported loss of Pax-2 protein expression with EH and endometrial carcinogenesis.^{25, 38} *Bcl-2* is an antiapoptotic gene that suppresses programmed cell death and is expressed in a variety of human neoplasms.³⁹ Previous studies suggested that Bcl-2 is expressed in the early stage of the neoplastic continuum³⁹⁻⁴² and is down-regulated with administration of progesterone.^{19, 22}

There are strengths and limitations of our study. Baseline and follow-up EH diagnoses were strictly adjudicated by blinded research pathologists,¹⁵ minimizing potential diagnostic misclassification.¹⁵ This allowed analyses to be stratified by EH type (CH, AH) to elucidate associations that may be missed by grouping together women with potentially different disease pathways and likelihood for cancer progression. Some limitations are similar to those of previous publications. First, it is possible that women with an index diagnosis of

AH had concomitant endometrial carcinoma that was not detected by endometrial sampling.⁴³ Treatment decisions in clinical practice are therefore made, at times, without knowledge of concurrent existence of endometrial carcinoma. Guidelines indicate that atypical hyperplasia and grade 1 adenocarcinoma may be treated with progestin therapy if conservation of the uterus and fertility is desired.⁴⁴ Biomarkers predictive of progestin therapy resistance in the setting of atypical hyperplasia or endometrial carcinoma would be even more clinically valuable than biomarkers predictive of progestin therapy resistance in atypical hyperplasia alone. Thus, this is potentially a benefit of our study design, not a limitation. Second, this was not a randomized controlled trial of women with EH and, despite collecting 20 years of data, the study inclusion criteria requiring progestin therapy and the availability of two biopsies within a 6 month time frame resulted in a limited sample. The sample size affected our study power and the precision of the estimates of the associations, as seen by wide confidence intervals. However, unlike the majority of prior studies which reported *P*-values, we additionally reported odds ratios and 95% confidence intervals for transparency about statistical instability, and to provide the magnitude of the association. Third, IHC interpretations are prone to subjective bias, specifically in assigning a value for the percent of cells staining. To overcome this limitation and minimize exposure misclassification, we used a binary categorization of biomarker expression with a conservative threshold for high expression and no loss. Fourth, cases with AH had a shorter mean duration of progestin use and were more likely to receive lower progestin dose than controls. While progestin duration and dose would not confound our results since progestin treatment occurred after index biopsy, the specimen used for biomarker measurement, it is possible that the associations between individual biomarkers and progestin therapy resistance may vary by progestin duration and dose. The similar results observed among women treated with progestin for at least 8 weeks compared to those of the main analysis, minimize this concern. Fifth, endometrial hyperplasia is increasingly being treated with intrauterine levonorgestrel. However, the larger number of women with atypical hyperplasia in our study compared to studies of intrauterine levonorgestrel use,^{18, 20, 21} and the continued use of oral progestins in practice,⁴⁵ highlight the relevance of our study. Lastly, we did not evaluate, in the long-term, whether expression of biomarkers like PRB persist after progestin therapy is stopped.

The need persists for biomarkers that can serve the clinically important role of identifying which women with EH will respond to progestin treatment and warrants further research. While PRB expression shows promise as an easily evaluable biomarker of progestin response in women with AH, these results should be confirmed in adequately powered studies to better understand how PRB expression may guide treatment decisions.

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References

1. Altekruse, S.; Kosary, C.; Krapcho, M., et al. SEER Cancer Statistics Review 1975–2007. Bethesda, MD: National Cancer Institute; 2009. SEER Stat Fact Sheets: Corpus and Uterus, NOS.
2. Kurman RJ, Kaminski PF, Norris HJ. The behavior of endometrial hyperplasia. A long-term study of "untreated" hyperplasia in 170 patients. *Cancer*. 1985; 56:403–412. [PubMed: 4005805]

3. Lacey JV Jr, Ioffe OB, Ronnett BM, et al. Endometrial carcinoma risk among women diagnosed with endometrial hyperplasia: the 34-year experience in a large health plan. *Br J Cancer*. 2008; 98:45–53. [PubMed: 18026193]
4. Lacey JV Jr, Sherman ME, Rush BB, et al. Absolute risk of endometrial carcinoma during 20-year follow-up among women with endometrial hyperplasia. *J Clin Oncol*. 2010; 28:788–792. [PubMed: 20065186]
5. Management of endometrial cancer. ACOG Practice Bulletin No. 65. American College of Obstetricians and Gynecologists. *Obstet Gynecol*. 2005; 106:413–425. [PubMed: 16055605]
6. Clark TJ, Neelakantan D, Gupta JK. The management of endometrial hyperplasia: an evaluation of current practice. *Eur J Obstet Gynecol Reprod Biol*. 2006; 125:259–264. [PubMed: 16246481]
7. Marsden DE, Hacker NF. Optimal management of endometrial hyperplasia. *Best Pract Res Clin Obstet Gynaecol*. 2001; 15:393–405. [PubMed: 11476561]
8. Tavassoli, FA. World Health Organization Classification of tumors: pathology and genetics of tumors of the breast and female genital organs. Lyon (France): IARC Press; 2003.
9. Trimble CL, Kauderer J, Zaino R, et al. Concurrent endometrial carcinoma in women with a biopsy diagnosis of atypical endometrial hyperplasia: a Gynecologic Oncology Group study. *Cancer*. 2006; 106:812–819. [PubMed: 16400639]
10. Reed SD, Newton KM, Garcia RL, et al. Complex hyperplasia with and without atypia: clinical outcomes and implications of progestin therapy. *Obstet Gynecol*. 2010; 116:365–773. [PubMed: 20664397]
11. Bergeron C, Nogales FF, Masseroli M, et al. A multicentric European study testing the reproducibility of the WHO classification of endometrial hyperplasia with a proposal of a simplified working classification for biopsy and curettage specimens. *Am J Surg Pathol*. 1999; 23:1102–1108. [PubMed: 10478671]
12. Kendall BS, Ronnett BM, Isacson C, et al. Reproducibility of the diagnosis of endometrial hyperplasia, atypical hyperplasia, and well-differentiated carcinoma. *Am J Surg Pathol*. 1998; 22:1012–1019. [PubMed: 9706982]
13. Skov BG, Broholm H, Engel U, et al. Comparison of the reproducibility of the WHO classifications of 1975 and 1994 of endometrial hyperplasia. *Int J Gynecol Pathol*. 1997; 16:33–37. [PubMed: 8986530]
14. Zaino RJ, Kauderer J, Trimble CL, et al. Reproducibility of the diagnosis of atypical endometrial hyperplasia: a Gynecologic Oncology Group study. *Cancer*. 2006; 106:804–811. [PubMed: 16400640]
15. Allison KH, Reed SD, Voigt LF, Jordan CD, Newton KM, Garcia RL. Diagnosing endometrial hyperplasia: why is it so difficult to agree? *Am J Surg Pathol*. 2008; 32:691–698. [PubMed: 18347507]
16. Reed SD, Voigt LF, Newton KM, et al. Progestin therapy of complex endometrial hyperplasia with and without atypia. *Obstet Gynecol*. 2009; 113:655–662. [PubMed: 19300331]
17. Allison KH, Tenpenny E, Reed SD, Swisher EM, Garcia RL. Immunohistochemical markers in endometrial hyperplasia: is there a panel with promise? A review. *Appl Immunohistochem Mol Morphol*. 2008; 16:329–343. [PubMed: 18528284]
18. Akesson E, Gallos ID, Ganesan R, Varma R, Gupta JK. Prognostic significance of estrogen and progesterone receptor expression in LNG-IUS (Mirena) treatment of endometrial hyperplasia: an immunohistochemical study. *Acta Obstet Gynecol Scand*. 2010; 89:393–398. [PubMed: 20199355]
19. Amezcua CA, Zheng W, Muderspach LI, Felix JC. Down-regulation of bcl-2 is a potential marker of the efficacy of progestin therapy in the treatment of endometrial hyperplasia. *Gynecol Oncol*. 1999; 73:126–136. [PubMed: 10094893]
20. Orbo A, Arnes M, Pettersen I, Larsen K, Hanssen K, Moe B. Down-regulated progesterone receptor A and B coinciding with successful treatment of endometrial hyperplasia by the levonorgestrel impregnated intrauterine system. *Acta Obstet Gynecol Scand*. 2010; 89:1438–1446. [PubMed: 20955098]
21. Vereide AB, Kaino T, Sager G, Arnes M, Orbo A. Effect of levonorgestrel IUD and oral medroxyprogesterone acetate on glandular and stromal progesterone receptors (PRA and PRB),

- and estrogen receptors (ER-alpha and ER-beta) in human endometrial hyperplasia. *Gynecol Oncol*. 2006; 101:214–223. [PubMed: 16325240]
22. Vereide AB, Kaino T, Sager G, Orbo A. Bcl-2, BAX, apoptosis in endometrial hyperplasia after high dose gestagen therapy: a comparison of responses in patients treated with intrauterine levonorgestrel and systemic medroxyprogesterone. *Gynecol Oncol*. 2005; 97:740–750. [PubMed: 15885761]
 23. Pecorelli S, Benedet JL, Creasman WT, Shepherd JH. FIGO staging of gynecologic cancer 1994–1997 FIGO Committee on Gynecologic Oncology. International Federation of Gynecology and Obstetrics. *Int J Gynaecol Obstet*. 1999; 65:243–249. [PubMed: 10428343]
 24. Reed SD, Newton KM, Clinton WL, et al. Incidence of endometrial hyperplasia. *Am J Obstet Gynecol*. 2009; 200:678, e1–e6. [PubMed: 19393600]
 25. Allison KH, Upson K, Reed SD, et al. PAX2 loss by immunohistochemistry occurs early and often in endometrial hyperplasia. *J Gyn Pathol*. 2011 Accepted.
 26. Hammond R, Johnson J. Endometrial hyperplasia. *Curr Obstet Gynaecol*. 2004; 14:99–103.
 27. Effects of hormone replacement therapy on endometrial histology in postmenopausal women. The Postmenopausal Estrogen/Progestin Interventions (PEPI) Trial. The Writing Group for the PEPI Trial. *JAMA*. 1996; 275:370–375. [PubMed: 8569016]
 28. Tseng L, Gurside E. Induction of human endometrial estradiol dehydrogenase by progestins. *Endocrinology*. 1975; 97:825–833. [PubMed: 172318]
 29. Graham JD, Clarke CL. Physiological action of progesterone in target tissues. *Endocr Rev*. 1997; 18:502–519. [PubMed: 9267762]
 30. Tseng L, Gurside E. Effects of progestins on estradiol receptor levels in human endometrium. *J Clin Endocrinol Metab*. 1975; 41:402–404. [PubMed: 169281]
 31. Dai D, Kumar NS, Wolf DM, Leslie KK. Molecular tools to reestablish progestin control of endometrial cancer cell proliferation. *Am J Obstet Gynecol*. 2001; 184:790–797. [PubMed: 11303185]
 32. Smid-Koopman E, Blok LJ, Kuhne LC, et al. Distinct functional differences of human progesterone receptors A and B on gene expression and growth regulation in two endometrial carcinoma cell lines. *J Soc Gynecol Investig*. 2003; 10:49–57.
 33. Utsunomiya H, Suzuki T, Ito K, et al. The correlation between the response to progestogen treatment and the expression of progesterone receptor B and 17beta-hydroxysteroid dehydrogenase type 2 in human endometrial carcinoma. *Clin Endocrinol (Oxf)*. 2003; 58:696–703. [PubMed: 12780745]
 34. Penner, KR.; Wadehra, M.; Steinhardt, AA., et al. Progesterone Receptors A and B, pAkt, and p4EBP1 Expression in Grade 1 Endometrial Adenocarcinoma (G1EAC) and Complex Atypical Hyperplasia (CAH) and Prediction of Response to Progestin Therapy. United States & Canadian Academy of Pathology (USCAP) 2011 Annual Meeting; San Antonio, TX. 2011.
 35. Kimura F, Watanabe J, Hata H, et al. PTEN immunohistochemical expression is suppressed in G1 endometrioid adenocarcinoma of the uterine corpus. *J Cancer Res Clin Oncol*. 2004; 130:161–168. [PubMed: 14689303]
 36. Guzeloglu-Kayisli O, Kayisli UA, Al-Rejjal R, Zheng W, Luleci G, Arici A. Regulation of PTEN (phosphatase and tensin homolog deleted on chromosome 10) expression by estradiol and progesterone in human endometrium. *J Clin Endocrinol Metab*. 2003; 88:5017–5026. [PubMed: 14557489]
 37. Minaguchi T, Nakagawa S, Takazawa Y, et al. Combined phospho-Akt and PTEN expressions associated with post-treatment hysterectomy after conservative progestin therapy in complex atypical hyperplasia and stage Ia, G1 adenocarcinoma of the endometrium. *Cancer Lett*. 2007; 248:112–122. [PubMed: 16919866]
 38. Monte NM, Webster KA, Neuberg D, Dressler GR, Mutter GL. Joint loss of PAX2 and PTEN expression in endometrial precancers and cancer. *Cancer Res*. 2010; 70:6225–6232. [PubMed: 20631067]
 39. Vaskivuo TE, Stenback F, Tapanainen JS. Apoptosis and apoptosis-related factors Bcl-2, Bax, tumor necrosis factor-alpha, and NF-kappaB in human endometrial hyperplasia and carcinoma. *Cancer*. 2002; 95:1463–1471. [PubMed: 12237915]

40. Kokawa K, Shikone T, Otani T, et al. Apoptosis and the expression of Bax and Bcl-2 in hyperplasia and adenocarcinoma of the uterine endometrium. *Hum Reprod.* 2001; 16:2211–2218. [PubMed: 11574518]
41. Peiro G, Diebold J, Baretton GB, Kimmig R, Lohrs U. Cellular apoptosis susceptibility gene expression in endometrial carcinoma: correlation with Bcl-2, Bax, caspase-3 expression and outcome. *Int J Gynecol Pathol.* 2001; 20:359–367. [PubMed: 11603220]
42. Sakuragi N, Salah-eldin AE, Watari H, et al. Bcl-2, and p53 expression in endometrial cancer. *Gynecol Oncol.* 2002; 86:288–296. [PubMed: 12217750]
43. Shutter J, Wright TC Jr. Prevalence of underlying adenocarcinoma in women with atypical endometrial hyperplasia. *Int J Gynecol Pathol.* 2005; 24:313–318. [PubMed: 16175074]
44. ACOG practice bulletin, clinical management guidelines for obstetrician-gynecologists, number 65, August 2005: management of endometrial cancer. *Obstet Gynecol.* 2005; 106:413–425. [PubMed: 16055605]
45. Gallos ID, Ofinran O, Shehmar M, Coomarasamy A, Gupta JK. Current management of endometrial hyperplasia-a survey of United Kingdom consultant gynaecologists. *Eur J Obstet Gynecol Reprod Biol.* 2011; 158:305–307. [PubMed: 21658836]

Table 1
Immunohistochemistry procedures for PRA, PRB, PTEN, Pax-2, and Bcl-2 protein expression.

Procedure	PRA	PRB	PTEN	Pax-2	Bcl-2
Pre-treatment	Citrate 18 minutes	Citrate 18 minutes	Citrate 18 minutes	EDTA 15 minutes	Citrate 15 minutes
Antibody	Mouse monoclonal	Mouse monoclonal	Mouse monoclonal	Rabbit Polyclonal	Mouse monoclonal
			Clone: 6H2-1	Clone: Z-RX2	Clone: 124
	Novocastra/Leica Microsystems, IL	Novocastra/Leica Microsystems, IL	Cascade Bioscience, MA	Zymed, CA	Cell Marque, CA
Dilution	1:8000	1:1000	1:100	1:100	1:25
Incubation time	40 Min at RT	40 Min at RT	40 Min at RT	40 Min at RT	40 Min at RT
Positive control	Normal proliferative endometrium	Normal proliferative endometrium	Normal proliferative endometrium	Normal endometrial stroma	Tonsil

Abbreviation: RT=room temperature.

Table 2

Characteristics of 114 women treated with progestin with complex hyperplasia with and without atypia who persisted or progressed (cases) or regressed (controls) after six months.

Characteristics	Complex (n=73)		Atypia (n=41)	
	Cases (n=21)	Controls (n=52)	Cases (n=12)	Controls (n=29)
Age (years)				
39	4 (19)	2 (4)	1 (8)	3 (10)
40–49	4 (19)	17 (33)	4 (33)	3 (10)
50–59	6 (29)	13 (25)	5 (42)	12 (41)
60–69	6 (29)	13 (25)	0 (0)	5 (17)
70	1 (5)	7 (13)	2 (17)	6 (21)
Caucasian ^a	18 (95)	45 (90)	8 (73)	20 (80)
Diabetes ^a	2 (11)	4 (8)	0 (0)	3 (12)
Personal history of breast or colon cancer ^a	1 (6)	1 (2)	1 (9)	1 (4)
Current smoker ^a	3 (16)	4 (9)	2 (18)	2 (8)
BMI (kg/m ²) ^a				
<25	3 (16)	16 (32)	2 (18)	7 (29)
25–29.9	7 (37)	7 (14)	3 (27)	7 (29)
30	9 (47)	27 (54)	6 (55)	10 (42)
Nulliparous ^a	2 (11)	10 (20)	2 (18)	10 (40)
Oral contraceptive ^b	2 (10)	1 (2)	0 (0)	0 (0)
HT ^{b,c}	3 (14)	6 (12)	2 (17)	3 (10)
Unopposed estrogen ^{b,d}	3 (14)	7 (13)	0 (0)	3 (10)
Progestin only ^b	1 (5)	1 (2)	0 (0)	0 (0)
Number weeks between index and follow-up biopsy, mean (SD)	15.6 (4.9)	16.8 (4.3)	12.8 (2.5)	17.0 (4.3)
Number weeks of progestin treatment between index and follow-up, mean (SD)	9.7 (5.4)	11.3 (5.2)	9.7 (3.7)	13.5 (5.2)
Percent of time on progestin treatment, mean (SD)	64.1 (30.0)	68.5 (27.7)	75.3 (24.3)	79.3 (21.1)
Progestin treatment dose ^e				
Low	4 (19)	6 (12)	2 (17)	1 (4)
Medium/high	12 (57)	33 (65)	6 (50)	23 (82)
Maximum	5 (24)	12 (24)	4 (33)	4 (14)
Progestin treatment type ^f				
MPA	11 (52)	22 (42)	5 (42)	12 (41)
MEGA	7 (33)	21 (40)	5 (42)	13 (45)
NETA	0 (0)	6 (12)	2 (17)	1 (3)
Mixed class	3 (14)	3 (6)	0 (0)	3 (10)
Index biopsy year				
1985–1984	0 (0)	4 (8)	0 (0)	0 (0)
1990–1994	6 (29)	12 (23)	4 (33)	7 (24)
1995–1999	7 (33)	17 (33)	7 (58)	14 (48)

Characteristics	Complex (n=73)		Atypia (n=41)	
	Cases (n=21)	Controls (n=52)	Cases (n=12)	Controls (n=29)
2000–2005	8 (38)	19 (37)	1 (8)	8 (28)

Data are n(%).

Abbreviations: BMI=body mass index; HT=postmenopausal hormone therapy; SD=standard deviation; MPA=medroxyprogesterone acetate; MEGA-megestrol acetate, NETA=norethindrone acetate.

^aMissing data on: complex: race, diabetes, BMI - 4; history of breast/colon cancer, parity - 5; smoking - 7; atypia: race, diabetes, history of breast/colon cancer, smoking, parity - 5, BMI - 6.

^bDispensed in the six months preceding diagnosis of endometrial hyperplasia and dispensed for at least 2 months.

^cPostmenopausal estrogen plus progestin hormone therapy where progestin was dispensed for at least 1/3 of time that estrogen was dispensed.

^dPostmenopausal estrogen therapy where estrogen dispensed alone or with progestin if progestin was dispensed less than 1/3 of time that estrogen was dispensed.

^eLow dose=MPA <10mg/day, NETA <1mg/day; Medium/high dose=MPA 10mg/day, NETA 1mg/day, MEGA<40mg/day; Maximum=MEGA 40mg/day.

^fProgestin type received 80% days progestin dispensed; Mixed type if several types of progestin dispensed and predominant progestin dispensed <80% days or combined oral contraceptive dispensed.

Table 3

Biomarker expression at index biopsy among 114 women treated with progesterin with complex hyperplasia with and without atypia who persisted/progressed (cases) or regressed (controls) within six months.

Biomarkers	Complex (n=73)				Atypia (n=41)			
	Cases		Controls		Cases		Controls	
	(n=21)	(n=52)	(n=12)	(n=29)	(n=12)	(n=29)	(n=12)	(n=29)
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
PRA								
Low expression	5 (28)	19 (43)	1.0	Reference	6 (55)	7 (30)	1.0	Reference
High expression	13 (72)	25 (57)	2.0	(0.5–8.3)	0.390	5 (45)	16 (70)	0.4 (0.06–2.0)
PRB								
Low expression	6 (32)	15 (34)	1.0	Reference	8 (73)	6 (25)	1.0	Reference
High expression	13 (68)	29 (66)	1.1	(0.3–4.3)	1.000	3 (27)	18 (75)	0.1 (0.01–0.8)
PRA and PRB ^a								
PRA low, PRB low	5 (28)	13 (30)	1.0	Reference	6 (55)	4 (17)	1.0	Reference
PRA low/PRB high, PRA high/PRB low	1 (6)	8 (18)	0.3	(0.03–3.6)	2 (18)	4 (17)	0.3	(0.03–3.2)
PRA high, PRB high	12 (67)	23 (52)	1.4	(0.4–4.8)	0.464	3 (27)	15 (65)	0.1 (0.02–1.0)
PTEN								
No Loss	6 (30)	27 (52)	1.0	Reference	4 (33)	4 (14)	1.0	Reference
Loss	14 (70)	25 (48)	2.5	0.8–9.2	0.118	8 (67)	24 (86)	0.3 (0.05–2.3)
Pax-2								
No Loss	3 (14)	3 (6)	1.0	Reference	0 (0)	2 (7)	1.0	Reference
Loss	18 (86)	48 (94)	0.4	(0.05–3.1)	0.348	12 (100)	26 (93)	NE
Bcl-2								
No Loss	13 (62)	23 (46)	1.0	Reference	2 (18)	8 (28)	1.0	Reference
Loss	8 (38)	27 (54)	0.5	(0.2–1.7)	0.300	9 (82)	21 (72)	1.7 (0.3–19.5)

Data are n(%). Numbers do not add to total due to missing.

Abbreviations: OR=Odds ratio; 95%CI=95% Confidence Interval; NE=Not estimable.

^a Analysis restricted to women for whom data on both PRA and PRB expression were available.

Odds ratio and exact 95% confidence intervals estimated using epitab in STATA. P-value from Fisher's exact test.